

Fiat lux!

Phylogeny and Bioinformatics shed light on GABA functions in plants

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The non-protein amino acid γ -aminobutyric acid (GABA) accumulates in plants in response to a wide variety of environmental cues. Recent data point toward an involvement of GABA in tricarboxylic acid (TCA) cycle activity and respiration, especially in stressed roots. To gain further insights into potential GABA functions in plants, phylogenetic and bioinformatic approaches were undertaken. Phylogenetic reconstruction of the GABA transaminase (GABA-T) protein family revealed the monophyletic nature of plant GABA-Ts. However, this analysis also pointed to the common origin of several plant aminotransferases families, which were found more similar to plant GABA-Ts than yeast and human GABA-Ts. A computational analysis of *AtGABA-T* co-expressed genes was performed in roots and in stress conditions. This second approach uncovered a strong connection between GABA metabolism and glyoxylate cycle during stress. Both in silico analyses open new perspectives and hypotheses for GABA metabolic functions in plants.

Gamma-aminobutyric acid (GABA), a four-carbon non-protein amino acid, has drawn much attention since its first identification over 60 y ago in potato tuber,¹ rat brain² and yeast extract.³ Subsequent investigations of GABA functions followed two main directions: signaling and metabolism. Signaling effects of GABA have been intensively tackled in mammalian models. It has been clearly established that GABA acts as a neurotransmitter⁴ and impacts the central nervous system development.^{5,6} In plants, early studies in the 70–80s reported rapid GABA accumulation in response to many environmental cues.^{7–10} Based on these observations, it has been hypothesized that GABA could participate in stress responses and tolerance. Nevertheless, for decades, GABA functions in plants remained elusive. Major progress came along with the genomic era and availability of the powerful embedded tools. Genetic analyses in *Arabidopsis* demonstrated that GABA acts as a signal molecule for pollen tube guidance and elongation.^{11,12} Alteration of tricarboxylic acid (TCA) cycle enzyme activities shed light on GABA metabolic function and stressed the tight connection between GABA and respiration.^{13–15}

The functional implication of GABA metabolism in *Arabidopsis* salt stress tolerance has recently been reported.^{16,17} The GABA transaminase *gaba-t/pop2-1* mutant root growth was shown hypersensitive to the ionic component of salt stress,¹⁶ confirming the long-standing hypothesis of GABA involvement in plant stress response. Metabolic data revealed that GABA bridges amino and organic acids metabolisms in roots under

saline conditions.^{16,17} Moreover, a genome-wide transcriptional analysis indicated that central carbon metabolism is altered upon the loss of GABA metabolic function during salt stress. Indeed, genes involved in remobilization of carbon reserves (i.e., sucrose and starch) were found higher expressed in the *gaba-t/pop2-1* mutant.¹⁷ However, despite this transcriptional upregulation, a decrease in soluble sugars was observed in the mutant,^{16,17} a response that might be a compensatory effect of enhanced glycolysis balancing the reduced succinate supply due to GABA metabolism defect.¹⁷ Here, GABA function was further explored by (1) investigating the phylogeny of the GABA transaminase (GABA-T) protein family, and (2) performing a computational analysis of *Arabidopsis* *GABA-T* co-expressed genes.

Phylogenetic Analysis of GABA-T Protein Family

GABA-T-like protein sequences from plants, human and yeast were retrieved from Phytozome (www.phytozome.org) and GenBank (www.ncbi.nlm.nih.gov/genbank) databases based on sequence homology to *Arabidopsis* GABA-T (AtGABA-T; At3g22200). Protein sequences were aligned using MEGA5 software (www.megasoftware.net) and MUSCLE algorithm. Subsequently, phylogeny was reconstructed using the Neighbor-joining method (Jones-Taylor-Thornton model). Human and yeast GABA-T sequences were used to root the tree. Likewise,

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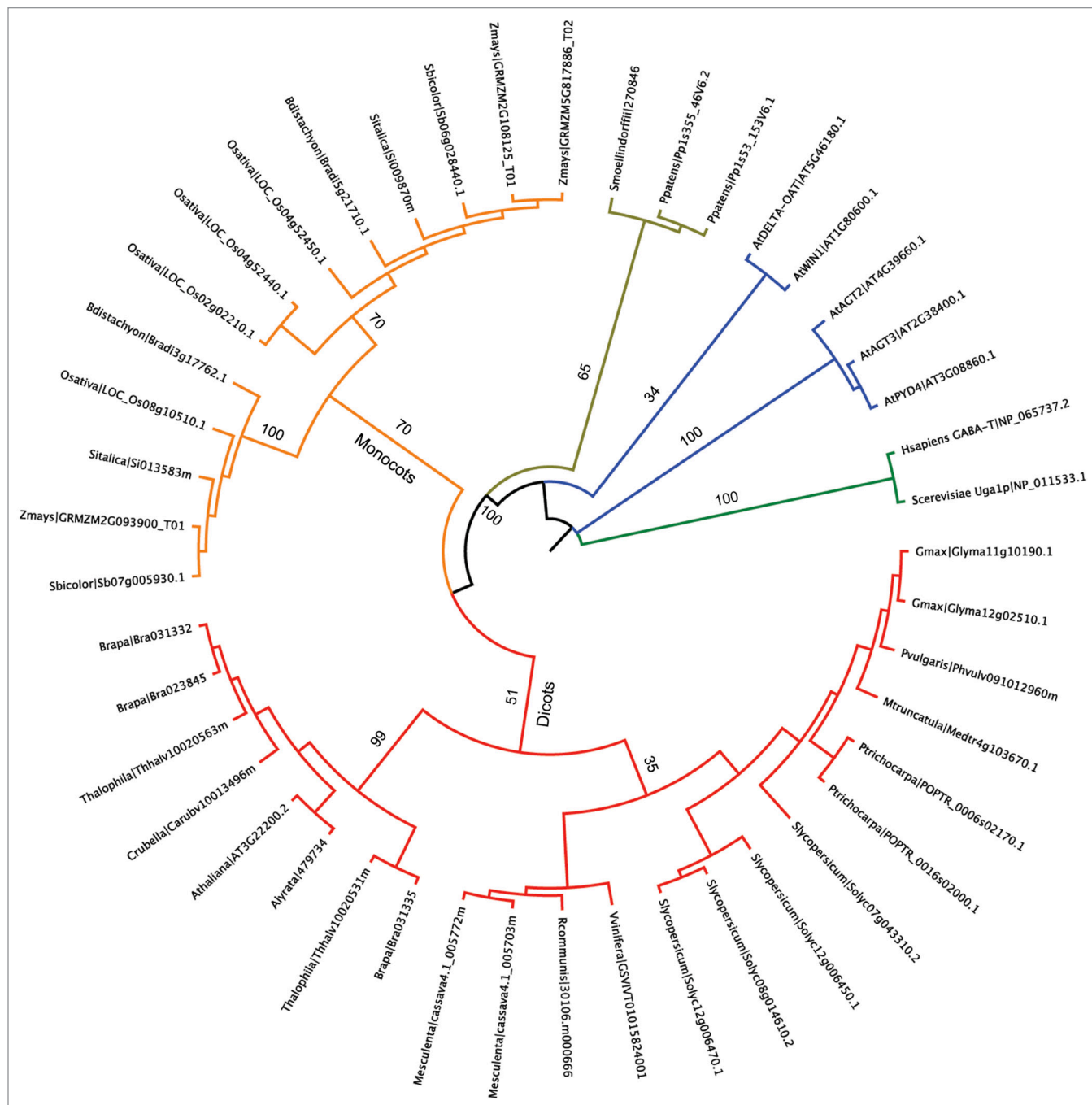


Figure 1. Phylogenetic relationships among plant GABA-T family. Neighbor-joining tree was constructed using the MEGA5 software. Human and yeast GABA-T protein sequences were used to root the tree. A set of Arabidopsis proteins closely related to AtGABA-T was included as outgroup. Branch lengths are proportional to phylogenetic distance; bootstrap values (expressed as percentage) from 1,000 iterations are indicated on main branches. For each protein, organism name and locus identifier/GenBank accession is provided.

a set of *Arabidopsis* aminotransferases closely related to AtGABA-T [i.e., alanine:glyoxylate aminotransferases (AGT2 and AGT3), β -alanine aminotransferase (PYD4), ornithine delta-aminotransferase (DELTA-OAT) and acetylornithine transaminase (WIN1)] served as outgroup. The phylogenetic reconstruction clearly discriminated GABA-Ts from ancestors (i.e., *Physcomitrella patens* and *Selaginella moellendorffii*), monocot and dicot lineages. Furthermore, an early and

conserved duplication of GABA-Ts was uncovered in monocots while in dicots only closely related paralogs evolved in some species (Fig. 1). Strikingly, the phylogenetic distance between the *Arabidopsis* aminotransferases and plant GABA-Ts was shorter than the distance between the functional yeast, human and plant GABA-T homologs (Fig. 1). This suggests that plant aminotransferases might share common substrates and activities. Arabidopsis, tomato and rice GABA-Ts, able to use both

pyruvate and glyoxylate as amino group acceptor,¹⁸⁻²⁰ exemplify the potentially broad substrate range of this class of proteins.

Functional Classification of *AtGABA-T* Co-expressed Genes

As *AtGABA-T* plays a prominent role in the metabolism of stressed roots,^{16,17} *AtGABA-T* co-expressed genes were identified in “AtGenExpress Root” and “AtGenExpress Stress” transcriptomic series using the Expression Angler software.²¹ Applying a stringent Pearson correlation coefficient threshold of 0.8, 254 and 140 genes co-expressing with *AtGABA-T* were retrieved from “Root” and “Stress” data sets, respectively (lists in **Supplemental data**). Among them, the expression of 12 genes correlated with *AtGABA-T* expression in both data sets (list “Root and Stress” in **Supplemental data**). Next, a functional analysis of the three gene sets (i.e., “Root,” “Stress,” “Root and Stress”) was performed using the Classification SuperViewer software²² and MapMan terms. Interestingly, “Polyamine metabolism,” “N metabolism” and “TCA org. transformation” terms were found more than 10-fold enriched among *AtGABA-T* co-expressed genes in the “Root” series (Table 1A). This result is in agreement with the previous finding that GABA links N and C metabolisms in roots.^{16,17} Other significantly enriched terms were associated with sugars (i.e., CHO), nucleotide and amino acid metabolisms, as well as with transport process and mitochondrial electron transport (Table 1A). The latter observation further corroborates the previously suggested participation of GABA metabolism to root respiration.¹⁷ In the gene list extracted from the “Stress” data set, “Gluconeogenesis/glyoxylate” term was almost 37-fold enriched compared with the entire genome (Table 1B). Strikingly, the same term was recovered as 200 times overrepresented in the overlap between “Root” and “Stress” series (Table 1C). The glyoxylate cycle allows to bypass the decarboxylation steps of TCA cycle and to use lipids as respiratory substrate or carbon source for gluconeogenesis.^{23,24} Regulation of the glyoxylate cycle genes is tuned by the carbon metabolic status of plants; the glyoxylate cycle is in particular induced under sugar starvation conditions.²⁵ Interestingly, roots of the Arabidopsis *gaba-t/pop2-1* mutant are deprived in sugars after salt stress,^{16,17} a similar phenomenon is observed in plants lacking glyoxylate cycle under low light,^{26,27} two conditions known to lead to limited carbon availability. These observations raise intriguing hypotheses for GABA function in plants, especially regarding stress acclimation. The glyoxylate cycle is initiated by the isocitrate lyase that cleaves isocitrate into glyoxylate and succinate in peroxysomes. Given that *AtGABA-T* can use glyoxylate as amino group acceptor,¹⁸ it can be hypothesized that it uses directly glyoxylate derived from isocitrate cleavage to form glycine and, after the succinic semialdehyde dehydrogenase step of GABA metabolism, a second succinate molecule. Alternatively, GABA metabolism could be embedded in a fine tuned metabolic network involving lipid and protein respiration that would allow stressed plants to keep fuelling cells with energy upon reduced carbon fixation. In support of second

Table 1. Functional classification of *AtGABA-T* co-expressed genes

MapMan term	Frequency	p-value
A. Root series (254 co-expressed genes)		
Polyamine metabolism	16.5	6.2E-03
N-metabolism	15.2	9.4E-04
TCA/org. transformation	11.5	2.4E-06
Co-factor and vitamin metabolism	6.5	3.0E-03
Nucleotide metabolism	5.9	6.4E-05
Minor CHO metabolism	5.3	2.2E-03
Amino acid metabolism	5.1	2.6E-05
Major CHO metabolism	3.9	3.5E-02
Transport	3.7	1.6E-09
Mitochondrial electron transport/ATP synthesis	3.4	2.5E-02
B. Stress series (140 co-expressed genes)		
Gluconeogenesis/glyoxylate	36.8	1.3E-03
Nucleotide metabolism	8.0	1.0E-04
Lipid metabolism	6.3	1.3E-06
TCA/org. transformation	6.0	4.0E-02
Protein	1.3	2.7E-02
C. Genes common to both lists (12 genes)		
Gluconeogenesis/glyoxylate	214.8	4.6E-03
TCA/org. transformation	34.9	2.8E-02
Minor CHO metabolism	22.5	4.3E-06

AtGABA-T co-expressed genes were retrieved from “AtGenExpress Root” and “AtGenExpress Stress” transcriptomic series (Pearson correlation coefficient ≥ 0.8) available on the Expression Angler website. Genes co-expressed with *AtGABA-T* in “Root” (A), “Stress” (B) and in the overlap of both series (C) were functionally classified using the Functional SuperViewer software and MapMan terms. Only terms with a p-value below 0.05 are displayed.

hypothesis, the term “Lipid metabolism” was found six times enriched among *AtGABA-T* co-expressed genes under stress conditions (Table 1B).

Phylogenetic and bioinformatic data presented in this paper provide a platform to propose novel hypotheses of GABA functions in plants. In this context, it would be informative to experimentally explore the connection between GABA metabolism and glyoxylate cycle under stress conditions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental material may be found here:
www.landesbioscience.com/journals/psb/article/24274

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